

WHAT IS CLAIMED IS:

1. A method for cloning or subcloning one or more desired nucleic acid molecules comprising

(a) combining *in vitro* or *in vivo*

(i) one or more Insert Donor molecules comprising one or more desired nucleic acid segments flanked by at least two recombination sites, wherein said recombination sites do not substantially recombine with each other;

(ii) one or more Vector Donor molecules comprising at least two recombination sites, wherein said recombination sites do not substantially recombine with each other; and

(iii) one or more site-specific recombination proteins;

(b) incubating said combination under conditions sufficient to transfer one or more of said desired segments into one or more of said Vector Donor molecules, thereby producing one or more desired Product nucleic acid molecules;

(c) combining *in vitro* or *in vivo*

(i) one or more of said Product molecules comprising said desired segments flanked by two or more recombination sites, wherein said recombination sites do not substantially recombine with each other;

(ii) one or more different Vector Donor molecules comprising two or more recombination sites, wherein said recombination sites do not substantially recombine with each other; and

(iii) one or more site-specific recombination proteins; and

(d) incubating said combination under conditions sufficient to transfer one or more of said desired segments into one or more different Vector Donor molecules, thereby producing one or more different Product molecules.

2. ~~The method of claim 1, further comprising incubating said different Product molecules with one or more different Vector Donor molecules under conditions sufficient to transfer one or more of said desired segments into said different Vector Donor molecules.~~

3. A method for cloning or subcloning desired nucleic acid molecules comprising

a) combining *in vitro* or *in vivo*

i) one or more Insert Donor molecules comprising one or more nucleic acid segments flanked by two or more recombination sites, wherein said recombination sites do not substantially recombine with each other;

ii) two or more different Vector Donor molecules comprising two or more recombination sites, wherein said recombination sites do not substantially recombine with each other; and

iii) one or more site specific recombination proteins; and

b) incubating said combination under conditions sufficient to transfer one or more of said desired segments into said different Vector Donor molecules, thereby producing two or more different Product molecules.

4. The method of claim 1 or claim 3, wherein said Insert Donor molecules are derived from genomic DNA.

5. The method of claim 1 or claim 3, wherein said Insert Donor molecules are derived from cDNA.

6. The method of claim 1 or claim 3, wherein said Insert Donor molecules are produced by chemical synthesis.

7. The method of claim 1 or claim 3, wherein said Vector Donor molecules comprise at least one Selectable marker.

8. The method of claim 7, wherein the Selectable marker comprises at least one DNA segment selected from the group consisting of:

- (a) a DNA segment that encodes a product that provides resistance in a recipient cell against otherwise toxic compounds;
- (b) a DNA segment that encodes a product that is otherwise lacking in a recipient cell;
- (c) a DNA segment that encodes a product that suppresses the activity of a gene product in a recipient cell;
- (d) a DNA segment that encodes a product that can be identified;
- (e) a DNA segment that encodes a product that inhibits a cell function in a recipient cell;
- (f) a DNA segment that inhibits the activity of any of the DNA segments of (a)-(e) above;
- (g) a DNA segment that binds a product that modifies a substrate;
- (h) a DNA segment that encodes a specific nucleotide recognition sequence which can be recognized by a protein, an RNA, DNA or chemical.
- (i) a DNA segment that, when deleted, directly or indirectly confers sensitivity to cell killing by particular compounds within a recipient cell;
- (j) a DNA segment that encodes a product that is toxic in a recipient cell; and
- (k) a DNA segment that can be used to isolate or identify a desired molecule.

9. The method of claim 8, wherein said Selectable marker comprises at least one marker selected from the group consisting of an antibiotic resistance gene, a tRNA gene, an auxotrophic marker, a toxic gene, a phenotypic marker, an antisense oligonucleotide, a restriction endonuclease, a restriction endonuclease

cleavage site, an enzyme cleavage site, a protein binding site, and a sequence complementary to a PCR primer sequence.

10. The method of claim 1 or claim 3, wherein said Vector Donor molecules comprise prokaryotic and/or eukaryotic vectors.

11. The method of claim 10, wherein said eukaryotic vectors comprise vectors which propagate and/or replicate in yeast cells, plant cells, fish cells, eukaryotic cells, mammalian cells, and/or insect cells.

12. The method of claim 10, wherein said prokaryotic vectors comprise vectors which propagate and/or replicate in gram negative or gram positive bacteria.

13. The method of claim 12, wherein said prokaryotic vectors comprise vectors which propagate and/or replicate in bacteria of the genus *Escherichia*, *Salmonella*, *Bacillus*, *Streptomyces* and/or *Pseudomonas*.

14. The method of claim 13, wherein said prokaryotic vector comprises a vector which propagates and/or replicates in *E. coli*.

15. The method of claim 10, wherein said Vector Donor molecules are selected from the group consisting of cloning vectors, sequencing vectors, expression vectors, fusion vectors, 2-hybrid vectors, reverse 2-hybrid vectors or derivatives or variants thereof.

16. The method of claim 10, wherein said eukaryotic vectors are selected from the group consisting of pFastBac, pFastBac HT, pFastBac DUAL, pSFV, pTet-Splice, pEUK-C1, pPUR, pMAM, pMAMneo, pBI101, pBI121, pDR2, pCMVEBNA, YACneo, pSVK3, pSVL, pMSG, pCH110, pKK232-8, p3'SS, pXT1, pSG5, pPbac, pMbac, pMC1neo, and pOG44, pYES2, pAC360,

pBlueBacHis, pVL1392, pBlueBacIII, pCDM8, pcDNA1, pZeoSV, pcDNA3 pREP4, pCEP4, and pEBVHis or derivatives or variants thereof.

17. The method of claim 10, wherein said prokaryotic vectors are selected from the group consisting of pcDNA II, pSL301, pSE280, pSE380, pSE420, pTrcHis, pRSET, pGEMEX-1, pGEMEX-2, pET, pTrc99A, pKK223-3, pGEX, pEZZ18, pRIT2T, pMC1871, pKK233-2, pKK388-1, and pProEx-HT or derivatives or variants thereof.

18. The method of claim 15, wherein said 2-hybrid and reverse 2-hybrid vectors are selected from the group consisting of pPC86, pDBLeu, pDBTrp, pPC97, p2.5, pGAD1-3, pGAD10, pAct, pACT2, pGADGL, pGADGH, pAS2-1, pGAD424, pGBT8, pGBT9, pGAD-GAL4, pLexA, pBD-GAL4, pHISi, pHISi-1, placZi, pB42AD, pDG202, pJK202, pJG4-5, pNLexA, and pYESTrp or derivatives or variants thereof.

19. The method of claim 1 or claim 3, wherein said Insert Donor molecules comprise a vector.

20. The method of claim 1 or claim 3, wherein said Insert Donor molecules comprise a DNA segment produced by amplification.

21. The method of claim 20, wherein said amplification is PCR.

22. The method of claim 21, wherein said Insert Donor is linear.

23. The method of claim 22, wherein said Insert Donor comprises at least one recombination site at or near one or both termini of said linear molecule.

24. The method of claim 1 or claim 3, wherein said recombination sites are selected from the group consisting of loxP, attB, attP, attL, and attR.

25. ~~The method of claim 1 or claim 3, wherein said recombination proteins are selected from the group consisting of Int, Cre, Flp, Res.~~

26. A method for preparing a nucleic acid molecule comprising two or more recombination sites or portions thereof comprising

- (a) mixing a nucleic acid template with a polypeptide having polymerase activity and one or more primers comprising one or more recombination sites or portions thereof; and
- (b) incubating said mixture under conditions sufficient to synthesize a nucleic acid molecule which is complementary to all or a portion of said template and which comprises one or more recombination sites or portions thereof.

27. The method of claim 26, further comprising incubating said synthesized molecule in the presence of one or more primers comprising one or more recombination sites or portions thereof under conditions sufficient to synthesize a second nucleic acid molecule complementary to all or a portion to said first nucleic acid molecule, thereby producing a double stranded nucleic acid molecule comprising two or more recombination sites or portions thereof.

28. The method of claim 27, wherein said recombination sites or portions thereof are located at or near one or more termini of said synthesized double stranded nucleic acid molecule.

29. The method of claim 27, wherein said template is RNA or DNA.

30. The method of claim 29, wherein said RNA is an mRNA or a polyA RNA molecule.

31. The method of claim 27, wherein said polypeptide is selected from the group consisting of a reverse transcriptase or DNA polymerase.

Sub A2

Sub B2

Sub D3

Sub B3

356207 4824760

32. The method of claim 31, wherein said DNA polymerase is a thermostable DNA polymerase.

33. The method of claim 32, wherein said thermostable DNA polymerase is selected from the group consisting of *Thermus thermophilus* (Tth) DNA polymerase, *Thermus aquaticus* (Taq) DNA polymerase, *Thermatoga neopolitana* (Tne) DNA polymerase, *Thermatoga maritima* (Tma) DNA polymerase, *Thermococcus litoralis* (Tli or VENT®) DNA polymerase, *Pyrococcus furiosus* (Pfu or DEEPVENT®) DNA polymerase, *Pyrococcus woosii* (Pwo) DNA polymerase, *Bacillus sterothermophilus* (Bst) DNA polymerase, *Sulfolobus acidocaldarius* (Sac) DNA polymerase, *Thermoplasma acidophilum* (Tac) DNA polymerase, *Thermus flavus* (Tfl/Tub) DNA polymerase, *Thermus ruber* (Tru) DNA polymerase, *Thermus brockianus* (DYNAZYME®) DNA polymerase, *Methanobacterium thermoautotrophicum* (Mth) DNA polymerase, and mutants, variants and derivatives thereof.

34. The method of claim 27, further comprising amplifying said first and second nucleic acid molecules.

35. The method of claim 34, wherein said amplification is accomplished by a method comprising

- (a) contacting said first nucleic acid molecule with a first primer which is complementary to a portion of said first nucleic acid molecule, and a second nucleic acid molecule with a second primer which is complementary to a portion of said second nucleic acid molecule with a polypeptide having polymerase activity;
- (b) incubating said mixture under conditions sufficient to form a third nucleic acid molecule complementary to all or a portion of said first nucleic acid molecule and a fourth nucleic acid molecule complementary to all or a portion of said second nucleic acid molecule;

(c) denaturing said first and third and said second and fourth nucleic acid molecules; and

(d) repeating steps (a) through (c) one or more times,

wherein said first primer and/or said second primer comprise one or more recombination sites or portions thereof.

36. A method for amplifying a nucleic acid molecule comprising

(a) contacting a first nucleic acid molecule with a first primer which is complementary to a portion of said first nucleic acid molecule, and a second nucleic acid molecule with a second primer which is complementary to a portion of said second nucleic acid molecule with a polypeptide having polymerase activity;

(b) incubating said mixture under conditions sufficient to form a third nucleic acid molecule complementary to all or a portion of said first nucleic acid molecule and a fourth nucleic acid molecule complementary to all or portion of all or said portion of said second nucleic acid molecule;

(c) denaturing said first and third and said second and fourth nucleic acid molecule; and

(d) repeating steps (a) through (c) one or more times,

wherein said first primer and/or second primer comprise one or more recombination sites or portions thereof.

37. A method for producing one or more cDNA molecules or a population of cDNA molecules comprising

(a) mixing an RNA template or population of RNA templates with a reverse transcriptase and one or more primers wherein said primers comprise one or more recombination sites or portions thereof; and

(b) incubating said mixture under conditions sufficient to make a first DNA molecule complementary to all or a portion of said template,



thereby forming a first DNA molecule comprising one or more recombination sites or portions thereof.

38. The method of claim 37, further comprising incubating said first DNA molecule with one or more primers which comprise one or more recombination sites or portions thereof under conditions sufficient to make a second DNA molecule complementary to all or a portion of said first DNA molecule, thereby producing a double stranded DNA molecule which comprises one or more recombination sites or portions thereof.

39. The method of claim 38, wherein said double stranded DNA molecule is linear.

40. The method of claim 39, wherein said double stranded DNA molecule comprises one or more recombination sites or portions thereof at or near one or both termini of said double stranded DNA molecule.

41. A method for synthesizing one or more nucleic acid molecules comprising one or more recombination sites, said method comprising:

- (a) obtaining one or more linear nucleic acid molecules; and
- (b) contacting said molecules with one or more adapters which comprise one or more recombination sites or portions thereof under conditions sufficient to add one or more of said adapters to one or more termini of said linear nucleic acid molecule.

42. The method of claim 41, wherein said linear nucleic acid molecules are derived from genomic DNA.

43. The method of claim 41, wherein said linear nucleic acid molecules are derived from cDNA.

44. The method of claim 41, wherein said linear nucleic acid molecules are produced by mechanical or enzymatic techniques.

45. The method of claim 41, wherein said linear nucleic acid molecules are produced by digesting one or more nucleic acid molecules with one or more restriction endonucleases.

46. A method for adding one or more recombination sites or portions thereof to one or more nucleic acid molecules, said method comprising:

- (a) contacting one or more nucleic acid molecules with one or more integration sequences which comprise one or more recombination sites or portions thereof; and
- (b) incubating said mixture under conditions sufficient to incorporate said integration sequences into said nucleic acid molecules.

47. The method of claim 46, wherein said integration sequences are selected from the group consisting of transposons, integrating viruses, integrating elements, integrons and recombination sequences.

48. The method of claim 47, wherein said integration sequence is added to genomic DNA.

49. A product produced by the process of any one of claims 1, 3, 27, 37, 41, and 46.

50. The method of claim 1 or claim 3, wherein said segment is produced by chemical synthesis.

51. The method of claim 47, wherein said integration sequence is added to a vector.

add C2  
add NO1  
add F3  
add G2